

X-ray structure of factor VII zymogen and implications for long-range allosteric effects of tissue factor

C. Eigenbrot and M.H. Ultsch

Department of Protein Engineering, Genentech, Inc.
1 DNA Way, South San Francisco, California 94080 USA

Coagulation factor VII (FVII) initiates a cascade that stems blood loss through the generation of a fibrin clot. Each step in the cascade involves the conversion of a serine protease zymogen into its active form, until prothrombin is converted into thrombin. Thrombin cleaves fibrinogen to fibrin, activates platelets, and also starts an anti-coagulatory process when it combines with thrombomodulin to activate protein C. Full enzymatic activity of FVIIa arises in a complex with the cell-bound co-factor Tissue Factor (TF). The TF/FVIIa complex activates the biologically important substrates factor X (FX), factor IX (FIX), and FVII. Binding of the zymogen FVII to TF increases the rate of its conversion to FVIIa¹. Many enzymes are capable of inducing the conversion of FVII to FVIIa, with FXa probably the most important *in vivo*².

There is considerable interest in the exact nature of changes in FVII during its conversion from zymogen to fully active enzyme in the TF/FVIIa complex³. The activation of gastric serine proteases trypsin and chymotrypsin is structurally well characterized⁴⁻⁷. Cleavage of a single peptide bond releases a pro-peptide, generates the mature N-terminal residue (Ile16 in FVIIa, using the chymotrypsinogen numbering convention), and promotes structural changes that create the active enzyme. The structural changes arise in a contiguous collection of 4 peptide segments collectively termed the activation domain⁸. Principal among these peptide segments is the new N-terminus itself, which becomes buried with its nonpolar side chain in a hydrophobic environment and its charged α -amino nitrogen atom compensated by a salt bridge to a key Asp side chain from the catalytic active site. Three associated loop segments undergo changes that create the substrate binding cleft. For the structurally characterized zymogen/enzyme pairs, and for a large number of other homologous proteins, this activation scenario provides full enzymatic competence. For FVIIa, however, the 'activated' form exhibits poor amidolytic activity, is essentially devoid of proteolytic activity, and attains full activity only when associated with TF. The low activity of FVIIa has been ascribed to an incomplete conversion of its activation domain from zymogen form to enzyme form⁹⁻¹¹ because, for instance, the protease domain's N-terminal α -amino nitrogen is more susceptible to chemical modification in the absence of TF than in the presence of TF¹².

Potent anticoagulant peptides were derived from naive libraries displayed on M13 phage by selection for binding to TF/FVIIa, and are shown to bind at exosites on the protease domain. The "A" class of peptides binds in a region centered on Leu251. One such peptide (A-183) has facilitated crystallographic analysis of the previously unknown FVII zymogen structure.

FVII, in common with other trypsin-like molecules, consists of two distinct β -barrel domains. Consistent with studies of homologous zymogen/enzyme pairs, we find the large conformational differences between FVII and FVIIa are in the second β -barrel. Because peptide A-183 binds in

the first β -barrel, we consider the analyses of zymogen structural features and changes caused by A-183 to be largely separable.

Differences between zymogen FVII and activated FVIIa are more extensive than those in analogous systems where both structures are known. Zymogen FVII has no active site substrate binding cleft, the S1 sub-site residue Asp189 having moved 12 Å. The region of the protease domain contacted by TF is significantly different, and would prevent TF binding as it has been previously observed. The β -strand B2 (residues 153-162) in the zymogen is shifted by three amino acids toward the C-terminus relative to the complementary β -strand A2. In addition to changes in the TF binding region, this shift moves residue Glu154 into position for hydrogen bonds with residues 21 and 22. If such hydrogen bonds were also present in FVIIa, they would preclude formation of the important salt-bridge between Ile16 and Asp194. We propose that TF interaction with the FVIIa protease domain promotes the loss of hydrogen bonds between Glu154 and residues 21 and 22 and thereby favors the Ile16-Asp194 salt-bridge.

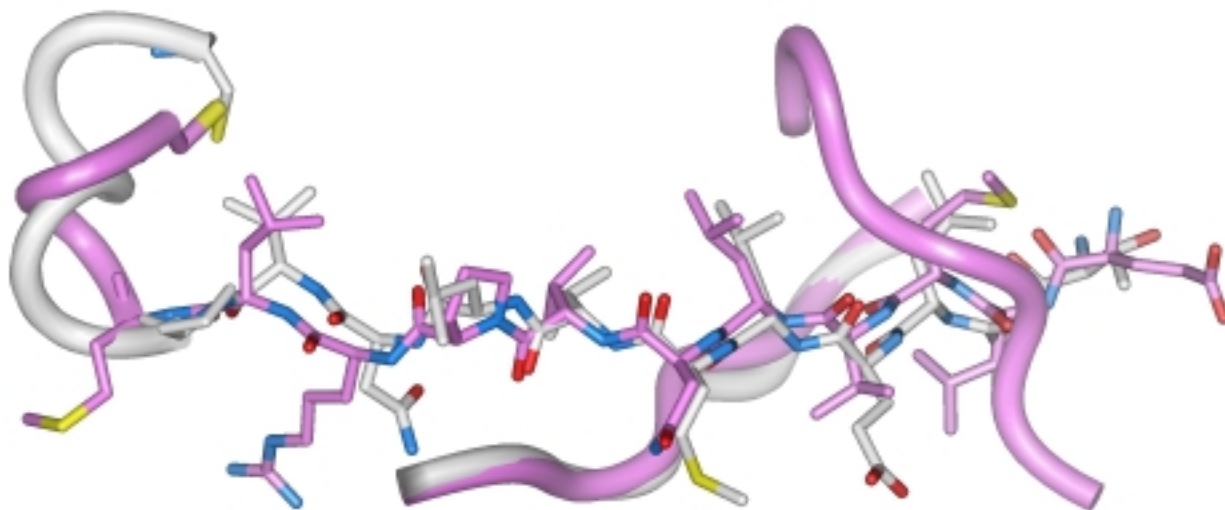


Figure 1. Superposition of β -strands B2 from FVIIa (pink) and FVII (white) The pink tube on the right represents N-terminal residues 16 to 23 from FVIIa which are not in this position in FVII. The overlapping pink and white tubes in the background represent β -strands A2 with which B2 shares characteristic β -sheet hydrogen bonds. During activation, extra residues on the left (in white) shift toward the right but maintain the secondary structure. Note the good correspondence of backbone atoms despite a three amino acid residue shift relative to each other.

ACKNOWLEDGEMENTS

We are grateful to Thomas Earnest and Keith Henderson of the Macromolecular Crystallography Facility at the Advanced Light Source for their assistance in data collection, and also our colleagues at Genentech D. Kirchhofer, R. Lazarus, M. Dennis, L. Santell, J. Stamos, and A.M. de Vos for their support.

REFERENCES

1. Nemerson, Y. & Repke, D. Tissue factor accelerates the activation of coagulation factor VII: the role of a bifunctional coagulation cofactor. *Thromb. Res.* **40**, 351-358 (1985).
2. Rao, L.V.M., Rapaport, S.I. & Bajaj, S.P. Activation of human factor VII in the initiation of tissue factor-dependent coagulation. *Blood* **68**, 685-691 (1986).
3. Banner, D.W., D'Arcy, A., Chene, C., Winkler, F.K., Guha, A., Konigsberg, W.H., Nemerson, Y. & Kirchhofer, D. The crystal structure of the complex of blood coagulation factor VIIa with soluble tissue factor. *Nature* **380**, 41-46 (1996).
4. Bode, W. & Huber, R. Crystal structure analysis and refinement of two variants of trigonal trypsinogen. *FEBS Lett.* **90**, 265-269 (1978).
5. Stroud, R.M., Kossiakoff, A.A. & L., C.J. Mechanisms of zymogen activation. *Annu. Rev. Biophys. Bioeng.* **6**, 177-193 (1977).
6. Bode, W., Fehlhhammer, H. & Huber, R. Crystal structure of bovine trypsinogen at 1.8 Å resolution. Data collection, application of Patterson search techniques and preliminary structural interpretation. *J. Mol. Biol.* **106**, 325-335 (1976).
7. Wang, D., Bode, W. & Huber, R. Bovine chymotrypsinogen A: x-ray crystal structure analysis and refinement of a new crystal form at 1.8Å resolution. *J. Mol. Biol.* **185**, 595-624 (1985).
8. Huber, R. & Bode, W. Structural basis of the activation and action of trypsin. *Acc. Chem. Res.* **11**, 114-122 (1978).
9. Higashi, S., Matsumoto, N. & Iwanaga, S. Molecular mechanism of tissue factor-mediated acceleration of factor VIIa activity. *J. Biol. Chem.* **271**, 26569-26574 (1996).
10. Dickinson, C.D., Kelly, C.R. & Ruf, W. Identification of surface residues mediating tissue factor binding and catalytic function of the serine protease factor VIIa. *Proc. Natl. Acad. Sci. USA* **93**, 14379-14384 (1996).
11. Kirchhofer, D. & Banner, D.W. Molecular and structural advances in Tissue Factor-dependent coagulation. *Trends Cardiovas. Med.* **7**, 316-324 (1997).
12. Higashi, S., Nishimura, H., Aita, K. & Iwanaga, S. Identification of regions of bovine factor VIIa essential for binding to tissue factor. *J. Biol. Chem.* **269**, 18891-18898 (1994).

This work was supported by Genentech, Inc.

Principal investigator: Charles Eigenbrot, Department of Protein Engineering, Genentech, Inc. 1 DNA Way, South San Francisco, California, 94080 USA. Email: charlie@gene.com. Telephone: 650-225-2106.